Increased Throughput in the Determination of PPCPs in Water Using Optimized MS Cycle Times in a High Sensitivity UHPLC-Triple Quadrupole System

Application Note

Environmental

Authors

Behrooz Zekavat, Thomas Glauner, Craig Marvin, André Santos, Tarun Anumol, and Jerry Zweigenbaum

Agilent Technologies, Inc.

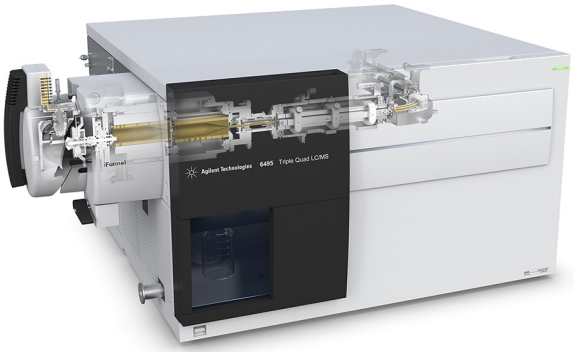
Santa Clara, CA

Introduction

Pharmaceuticals and personal care products (PPCPs) are contaminants in surface water. The presence of PPCPs in surface water has adverse effects on wildlife. Low concentrations of PPCPs pose significant analytical challenges for high-throughput analysis of these compounds using time-consuming solid phase extraction (SPE) prior to liquid chromatography/tandem mass spectrometry (LC/MS/MS). Moreover, PPCPs span a wide variety of compound classes and chemical properties. Therefore, it is necessary to use high-sensitivity MS instruments with both positive and negative ionization capability for comprehensive analysis in a high-throughput manner.

This Application Note evaluates modified software and firmware with optimized inter-MRM delay times in a triple quadrupole mass spectrometer equipped with an ion funnel for increased PPCP analysis throughput, while enabling direct injection of water samples without SPE.

We also show results, with high accuracy and precision, from the screening and quantitative analysis of 32 candidate PPCPs in surface water samples with concentrations as low as 0.5 ng/L.



Agilent Technologies

Experimental

Sample preparation

Neat standards of selected PPCPs (32 compounds from EPA Method 1694 and previously reported water contaminants) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). Calibration standards were prepared in Milli-Q water ranging from 0.1 ng/L to 1,000 ng/L. Three different source water samples, including surface water from two different rivers (Samples 2A and 3A) and one effluent sample from a sewage water treatment plant (Sample 4A) in Germany were analyzed quantitatively for PPCPs. Sample preparation consisted of filtering an aliquot of each sample through a 0.22mm filters into a sample vial. Afterwards, 40 µL were directly injected onto the LC/MS/MS system for analysis. The PPCPs were detected using MRM in polarity switching mode.

LC/MS/MS

UHPLC-MS/MS analyses were performed on an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6495 Triple Quadrupole Mass Spectrometer equipped with an Agilent Jet Stream (AJS) ion source.

Results and Discussion

Rapid LC/MS/MS analysis of PPCPs in water samples

Prepared PPCP standards were used for the initial optimization of the UHPLC/MS/MS method. Figure 1 shows the overlaid MRM chromatograms of the selected 32 PPCPs in water at 20 ng/L. Most of the compounds could be detected at concentrations as low as 0.5 ng/L without sample enrichment.

Table 1. Agilent 6495 Triple Quadrupole LC/MS, equipped with an Agilent Jet Stream Source conditions.

Parameter(s)	Setting
Drying gas temp. and flow	250 °C and 16 L/min
Nebulizer pressure	40 psi
Sheath gas temperature and flow	400 °C and 12 L/min
Capillary voltage	3 kV (pos)/3 kV (neg)

Table 2. Agilent 1290 Infinity UHPLC conditions.

Parameter	Setting
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm
Column temperature	60 °C
Injection volume	40 µL
Autosampler temperature	4 °C
Needle wash	15 seconds (80 % MEOH/20 % water)
Mobile phase	A) Water (0.03 % formic acid) B) Acetonitrile
Flow rate	0.5 mL/min
Solvent gradient (Post run = 1 minute)	Time (min) %B 0.0 15 0.5 15 6.0 60 6.1 100 6.4 100 6.5 15

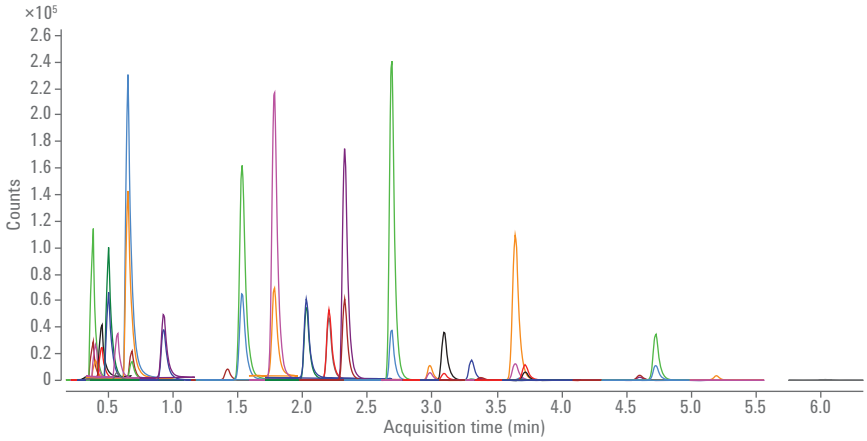


Figure 1. Overlaid MRM chromatograms of the selected PPCPs from 40 mL injection of a 20 ng/L mixture analyzed on an Agilent 6495 Triple Quadrupole LC/MS. The run finished within 6 minutes.

Most of the PPCP MRM transitions monitored could be detected at a 0.5 ms dwell time with minimum signal loss relative to a 5 ms dwell time. Figure 2 shows the overlaid MRM chromatograms of venlafaxine and carbamazepine at dwell times of 0.5 ms and 5.0 ms, collected using optimized inter-MRM delays. Data in Figure 2 show the importance of using appropriate inter-MRM delay times for maintaining the ion signal at short dwell times.

Accuracy and precision for analysis of PPCPs in water

The accuracy and precision of PPCPs were evaluated in the concentration range of 0.5 ng/L to 1,000 ng/L.

Figure 3 shows calibration plots for diclofenac (negative mode) and venlafaxine (positive mode) evaluated up to 11 standard concentrations ranging from the lowest limit of quantitation (LLOQ), as low as 0.5 ng/L, to the upper limit of quantitation (ULOQ), as high as 1 µg/ L. Accuracy and precision were calculated from 10 replicate injections at each level. Excellent assay precision (RSD% <10 % at LLOQ)) and average accuracy (85–112 %) were obtained.

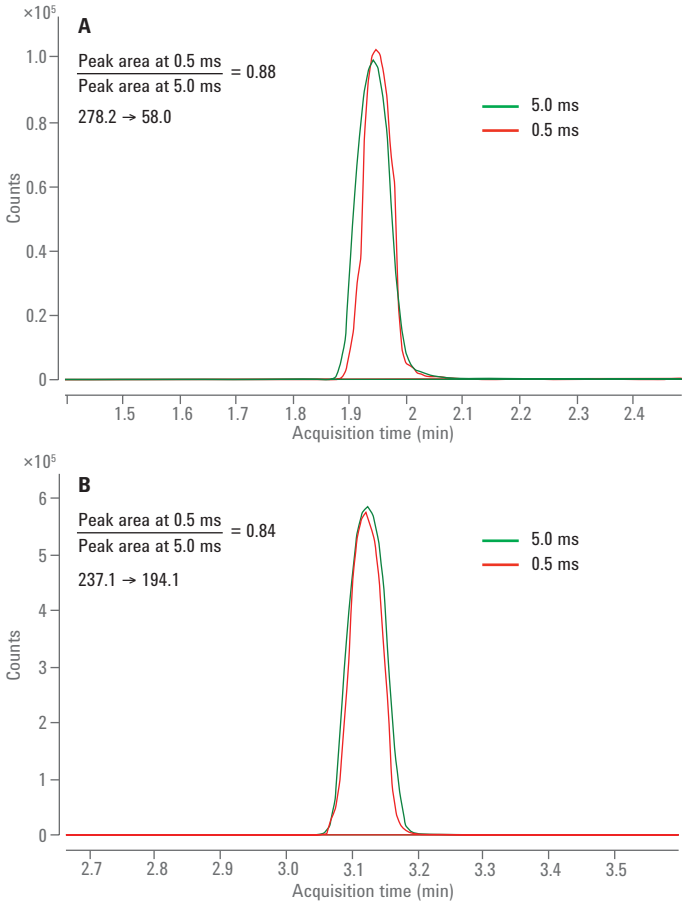


Figure 2. Overlaid MRM chromatograms of venlafaxine (A) and carbamazepine (B) (100 ng/L) at dwell times of 0.5 ms and 5.0 ms using optimized inter-MRM delay times.

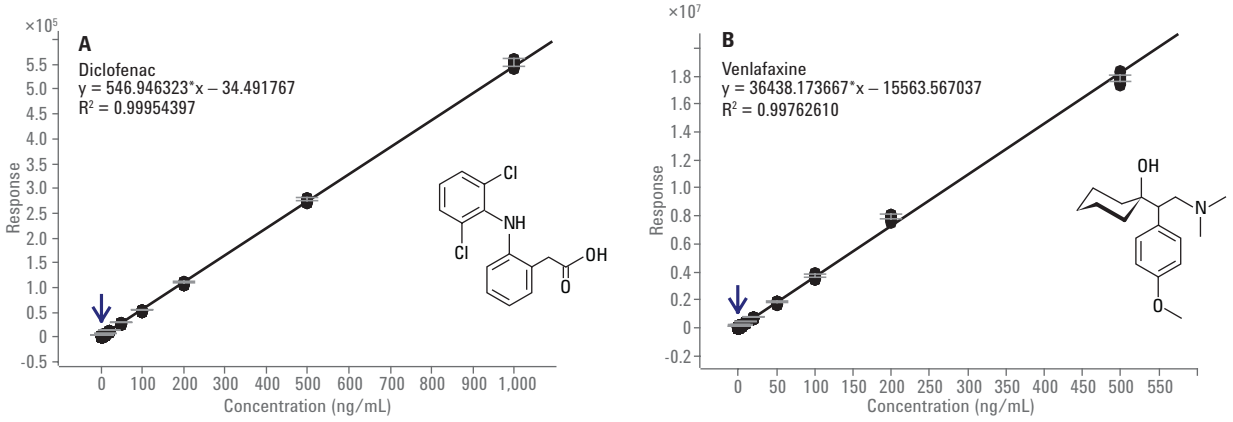


Figure 3. Calibration plots for diclofenac (negative mode) and venlafaxine (positive mode) in water evaluated for quantitation accuracy and peak area RSD.

Figure 4 shows the overlaid MRM chromatograms for two example PPCPs evaluated in this study at 0.5 ng/ L. Reproducible responses (RSD% <10 %) were observed for both quantifier and qualifier ions of atrazine (4A) and diltiazem (4B) at sub-ng/L concentrations of analytes.

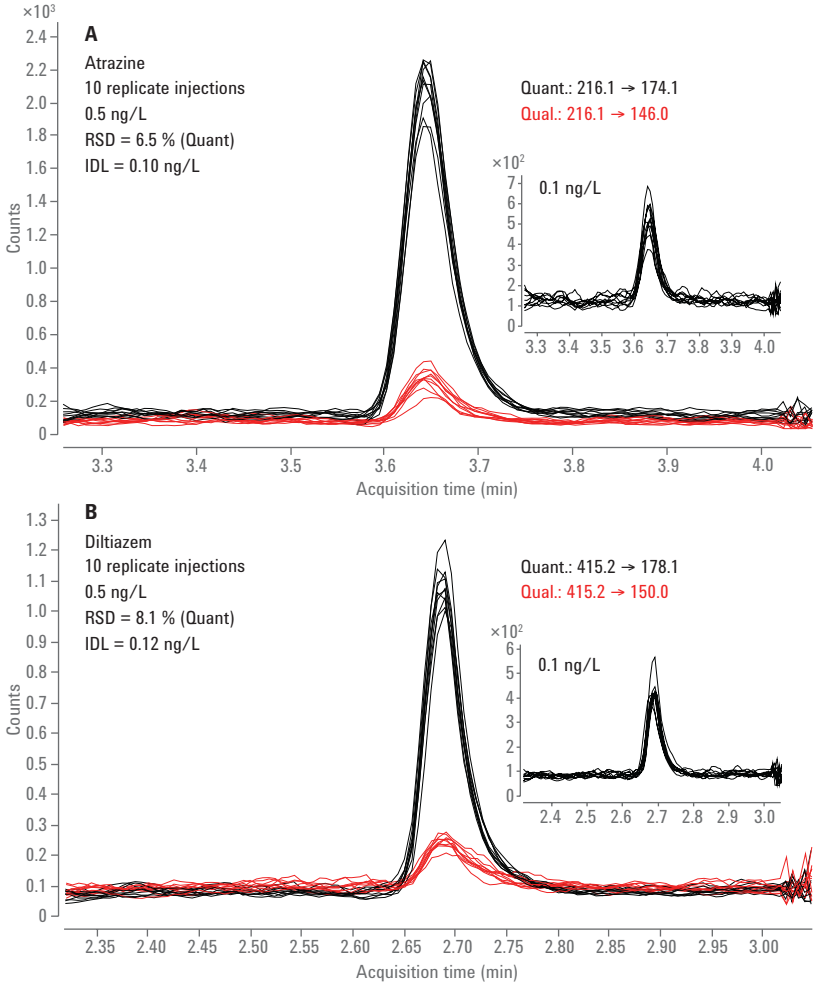


Figure 4. Overlaid MRM chromatograms of atrazine (A) and diltiazem (B) from 10 replicate injections at 0.5 ng/L level.

Screening PPCPs in surface water samples

Figure 5 shows the PPCP screening results from the analysis of three surface water samples using the UHPLC/MS/MS method presented in this work. Data in Figure 5 clearly show different PPCP profiles for the three water samples analyzed. This data may be used to identify the original source of the contamination in surface water or monitor the efficiency of water treatment plants.

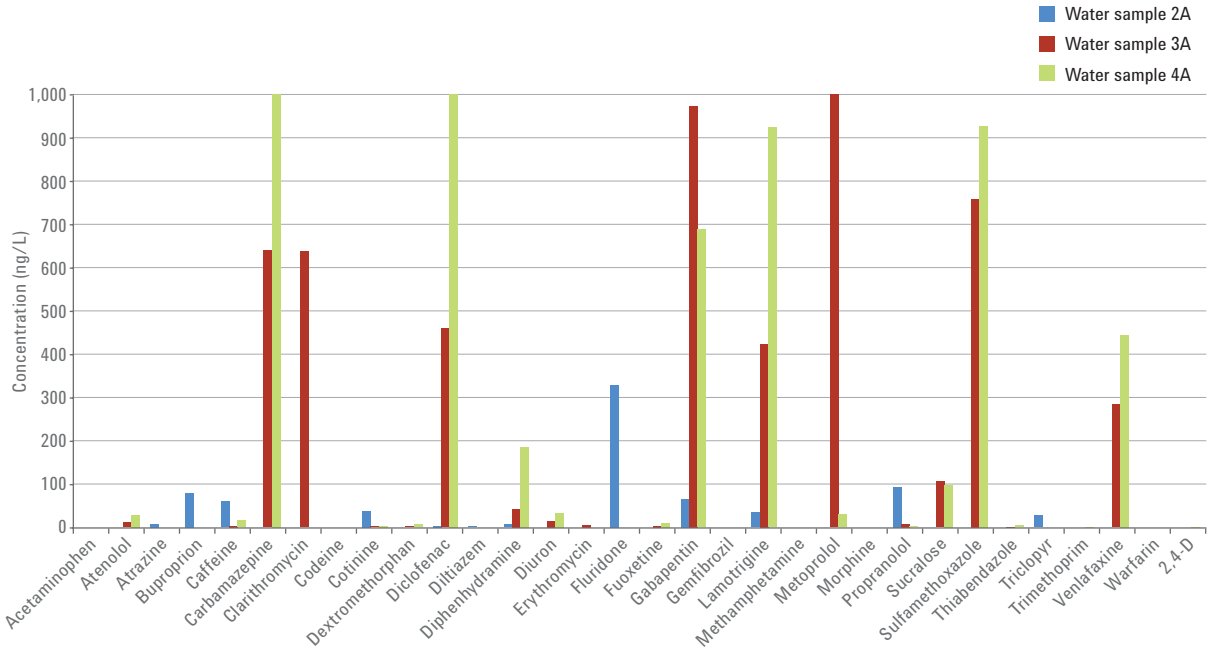


Figure 5. Summary of the PPCP screening results for three surface water samples.

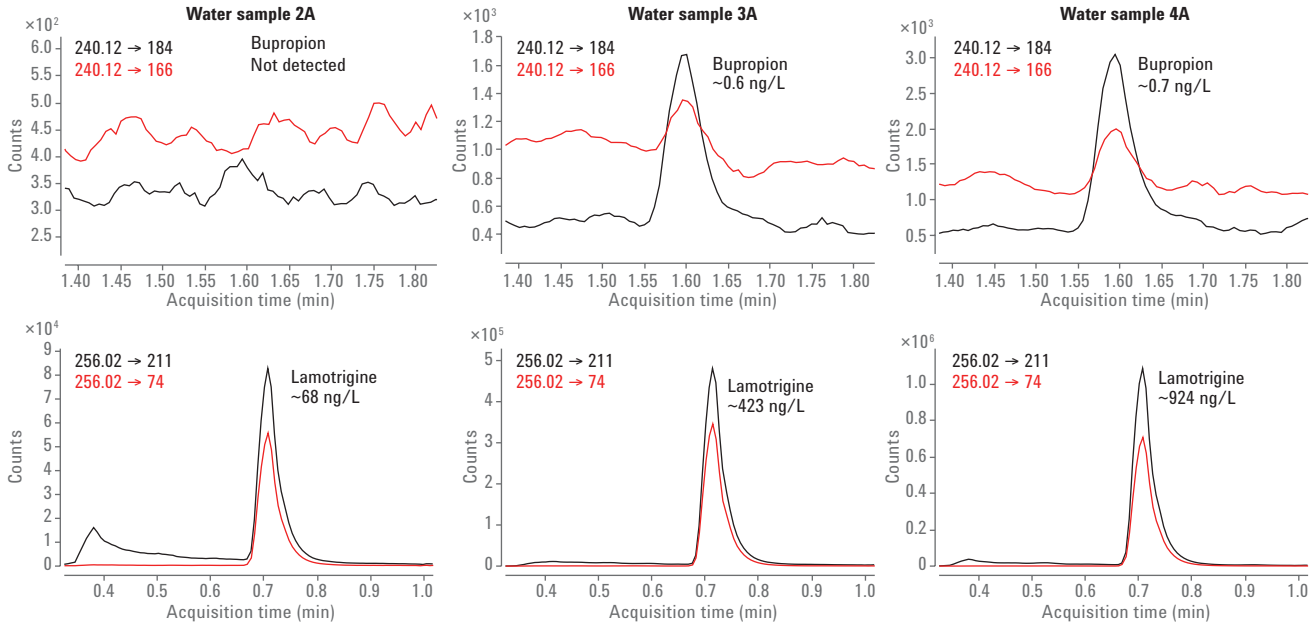


Figure 6. Examples of MRM chromatograms of two PPCPs detected in the analyzed surface water samples.

Conclusions

Optimized inter-MRM delay times allowed the use of dwell-times as short as 0.5 ms, with a minimum of analyte signal losses.

The combination of large volume injection (LVI), UHPLC, and short MRM dwell-times allowed high-throughput analysis of all 32 PPCPs within 6 minutes, with low limits of quantitation; an important consideration for surface water analysis.

The polarity switching UHPLC/MS/MS method reported in this work enables rapid screening of the candidate PPCPs ionizing in positive/negative ESI mode with high quantitative accuracy and precision in water samples without the need for time-consuming SPE preconcentration of analytes.

www.agilent.com/chem

Published in the USA, December 23, 2016
5991-7299EN

Agilent Technologies